

CLAIMS

What is claimed as the invention is:

1. Ovine tissue devoid of antibody-detectable Gal α (1,3)Gal determinants.
2. The tissue of claim 1, which is selected from the group consisting of lung tissue, kidney tissue, liver tissue, cardiac tissue, pancreatic tissue, and ocular tissue.
3. Isolated ovine cell or tissue that expresses glycosyl transferase enzymes but does not detectably express α (1,3)galactosyltransferase(α 1,3GT).
4. An ovine cell which is heterozygous or homozygous for inactivation of an α 1,3GT gene.
5. The cell of claim 4, produced by transfer of a nucleus from a donor cell heterozygous or homozygous for inactivation of an α 1,3GT gene, to an enucleated recipient cell.
6. An ovine animal that is homozygous for inactivation of an α 1,3GT gene.
7. A polynucleotide construct effective for inactivating an α 1,3GT gene in an ovine cell.
8. The polynucleotide construct of claim 7, comprising at least two polynucleotide sequences from an ovine α 1,3GT gene in a non-natural arrangement, for inactivating the α 1,3GT gene by homologous recombination.

9. The polynucleotide construct of claim 7, comprising an intron sequence of at least 30 consecutive nucleotides found in any of recombinant phage **B**, **C** and **G**, deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061.
10. The polynucleotide construct of claims 7, comprising a selectable marker.
11. The polynucleotide construct of claim 10, wherein the selectable marker is flanked on either side by polynucleotide sequences from an ovine $\alpha 1,3$ GT gene.
12. A method for inactivating an $\alpha 1,3$ GT gene in an ovine cell, comprising contacting the cell with the polynucleotide construct of claims 7 under conditions suitable for homologous recombination of the construct into the genome of the cell.
13. A method for producing an ovine that is homozygous for inactivation of an $\alpha 1,3$ GT gene, comprising providing an ovine embryo of cells according to claim 4, engrafting the embryo into the uterus of a female, birthing an ovine with an inactivated $\alpha 1,3$ GT gene from the engrafted female, and if the birthed ovine has the $\alpha 1,3$ GT gene inactivated on only one allele, then mating it with another ovine with an inactivated $\alpha 1,3$ GT gene, thereby producing an ovine that is homozygous for inactivation of the $\alpha 1,3$ GT gene.
14. A method for producing an isolated ovine cell that expresses glycosyl transferase enzymes but does not detectably express $\alpha 1,3$ GT, comprising isolating the cell from an ovine homozygous for inactivation of an $\alpha 1,3$ GT gene according to claim 6.

15. A method for producing ovine tissue devoid of antibody-detectable Gal α (1,3)Gal determinants, comprising harvesting the tissue from an ovine homozygous for inactivation of an α 1,3GT gene according to claim 6.
16. A method of xenotransplantation, comprising transplanting tissue devoid of antibody-detectable Gal α (1,3)Gal determinants according to claim 1 into a mammal having circulating antibody against Gal α (1,3)Gal determinants.
17. An isolated polynucleotide that comprises a sequence of at least 30 consecutive nucleotides with at least one of the following properties:
- a) it is contained in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13;
 - b) it is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; but not in λ -phage or any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13; or
 - c) it hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13
18. The isolated polynucleotide of claim 17, wherein said sequence is contained in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13.
19. The isolated polynucleotide of claim 17, wherein said sequence is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; but not in λ -phage or any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13.

20. The isolated polynucleotide of claim 17, wherein said sequence hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOs: 14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13.
21. The polynucleotide of claim 17, which is a construct effective for inactivating a $\alpha(1,3)$ galactosyltransferase ($\alpha(1,3)$ GT) gene in an ovine cell by homologous recombination.
22. An isolated polypeptide that comprises a sequence of at least 10 consecutive amino acids with at least one of the following properties:
- a) it is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12;
 - b) it is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ -phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12; or
 - c) it is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12
23. The isolated polypeptide of claim 22, wherein said sequence is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.
24. The isolated polypeptide of claim 22, wherein said sequence is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ -phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.

25. The isolated polypeptide of claim 22, wherein said sequence is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12.
26. The polypeptide of claim 22, which has glycosyltransferase activity.
27. An isolated polynucleotide comprising a sequence encoding a polypeptide according to claim 22.
28. An isolated polyclonal antibody or a monoclonal antibody that binds specifically to a polypeptide with the sequence SEQ. ID NO:2 but not to a peptide with the sequence present in any of SEQ. ID NOs: 4, 6, 8, or 10.
29. An assay method for determining α 1,3GT expression by a cell, comprising contacting a polynucleotide according to claim 17 with the cell or with mRNA or cDNA obtained from the cell, detecting any hybrids that form as a result, and correlating presence of the hybrids with expression of α 1,3GT by the cell.
30. A method for producing the antibody specific for sheep α 1,3GT, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide according to claim 22.
31. A method for preparing a Gal α (1,3)Gal determinant, comprising contacting a galactose acceptor saccharide with the polypeptide of claim 26 in the presence of UDP-galactose.

32. An assay method for determining $\alpha 1,3$ GT in a sample, comprising preparing a reaction mixture comprising the sample and an antibody according to claim 28 under conditions that permit the antibody to complex with $\alpha 1,3$ GT, and correlating any complex formed with the presence or amount of $\alpha 1,3$ GT in the sample.

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